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"RESPONSE UNDER 37 CFR 1.116-
EXPEDITED PROCEDURE EXAMINING
GROUP 1623

OBLON
SPIVAK
McCLELLAND
MAIER
&
NEUSTADT
P.C.

ATTORNEYS AT LAW

Docket No.: 218025US34PCT

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

RE: Application Serial No.: 09/125,022
Applicants: Silvio DE FLORA, et al.
Filing Date: NOVEMBER 24, 1998
For: PHARMACEUTICAL COMPOSITION ENABLING TO INHIBIT CANCER METASTASIS FORMATION CONTAINING N-ACETYL-CYSTEINE AND DOXORUBICIN
Group Art Unit: 1623
Examiner: Howard V. OWENS, Jr.

SIR:

Attached hereto for filing are the following papers:

Request for Reconsideration Under 37 CFR 1.116 (with Attachment)
Rule 132 Declaration (with (2) Attachments, A & B)
Notice of Appeal
Request for Extension of Time (two-month)
Information Disclosure Statement; PTO 1449; Statement of Relevancy; Cited References (3)

Our check in the amount of **$930.00** is attached covering any required fees. In the event any variance exists between the amount enclosed and the Patent Office charges for filing the above-noted documents, including any fees required under 37 C.F.R 1.136 for any necessary Extension of Time to make the filing of the attached documents timely, please charge or credit the difference to our Deposit Account No. 15-0030. Further, if these papers are not considered timely filed, then a petition is hereby made under 37 C.F.R. 1.136 for the necessary extension of time. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

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Norman F. Oblon
Registration No. 24,618

John T. Goolkasian
Registration No. 26,142

Customer Number
22850
(703) 413-3000 (phone)
(703) 413-2220 (fax)

218025US34PCT

"RESPONSE UNDER 37 CFR 1.116–
EXPEDITED PROCEDURE EXAMINING
GROUP 1623"

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF

DE FLORA, ET AL. : EXAMINER: HOWARD V. OWENS, JR.

SERIAL NO: 09/125,022 : GROUP ART UNIT: 1623

FILED: NOVEMBER 24, 1998 :

FOR: PHARMACEUTICAL COMPOSITION ENABLING TO INHIBIT CANCER METASTASIS FORMATION CONTAINING N-ACETYL-CYSTEINE AND DOXORUBICIN

REQUEST FOR RECONSIDERATION

COMMISSIONER FOR PATENTS
ALEXANDRIA, VA 22313

SIR:

Applicants hereby request reconsideration of the rejection set forth in the Office Action mailed July 15, 2003. The Office Action was designated a "Final Rejection" by the Examiner.

At this time, Applicants wish to acknowledge with appreciation the care and consideration the Examiner has given pending Claims 13 through 17. Applicants recognized that the prior art is somewhat close. However, Applicants firmly believe they are entitled to a patent for a new use of an old process.

As a general rule, doxorubicin is administered to patients in a later stage of cancer development. When doxorubicin is administered, the cancers have metastasized such that they are located both at the primary site but also at the site of metastasization. The reason for this late administration of doxorubicin is quite simple. As evidenced by the prior art relied on by the Examiner, doxorubicin is itself a deadly chemical which causes severe heart problems. Accordingly, doctors balance the deleterious effects of doxorubicin on the heart with the

beneficial effects of doxorubicin on the treatment of the metastasized cancer. The Doroshow and Freeman references relied on by the Examiner constitute an effort to learn, experimentally, whether N-acetylcysteine (NAC) would alleviate the adverse effects of doxorubicin on the animals' hearts.

The Doroshow and Freeman references do not mention metastasization and were not concerned with the prevention of metastasization. Rather, Doroshow and Freeman were concerned with the adverse effects of doxorubicin on the heart. Doroshow and Freeman reasoned that administering N-acetyl-cysteine in combination with doxorubicin would ameliorate and attenuate the adverse effects of doxorubicin. As set forth in the references, the heart is relatively incapable of eliminating free radicals. Free radicals are what cause deterioration of the heart. Hence, the idea was to use NAC, a free radical absorber, to cope with the free radicals induced by doxorubicin. The free radical absorbing effect of NAC was described by the authors as causing the extension of life in the tumor bearing mice.

The Examiner has taken the position that the suggestion in the Freeman and Doroshow references of using doxorubicin to treat "malignant" tumors also constitutes a suggestion to treat a subgroup of malignant tumors which are capable of metastasizing but wherein the tumor has not yet metastasized. This is simply not the case. Indeed, it would appear to be bad medicine. Logically, why would a physician risk heart problems with a patient bearing a tumor that has not yet spread. Accordingly, doxorubicin would not be suggested by the art for use on such non-metastasized tumors.

As evidenced by the Declaration of Dr. De Flora, the Freeman and Doroshow work is not concerned with metastasis. Indeed, to evaluate metastasis, one uses completely different experimental models. These experimental models are shown in Dr. De Flora's own paper of record in this case and the Budzynski et al. and Griswald et al. references, copies of which are submitted herewith.

It should be noted that the Griswold article, "Consideration of the Subcutaneously Implanted B16 Melanoma as a Screening Model for Potential Anticancer Agents," *Cancer Chemotherapy Report Part 2*, Vol. 3 No. 1, November 1972, confirms that there is a significant difference between the various screening tests for chemical compounds used to treat malignancies. The article notes that the B16 melanoma used by applicants is a model for solid tumors which metastasize whereas the L1210 ascites model is not. Indeed, with regard to this difference, the article states:

> None of these experimental tumors [L1210 ascites], however, adequately represent the spectrum of *solid* tumors in man where, in fact, the greatest part of the problem lies.

The Examiner takes the position that "it is inherent to administer an anti-cancer compound to a tumor to inhibit the growth of the tumor or eradicate the tumor so that metastasis does not occur." However, inherency cannot be obtained from the cited references because the tumors that are contained in the mice are not those which metastasize. Hence metastasization could not occur and "inherency" via prevention of metastasization does not exist.

The Examiner's "inherency" position would be appropriate if applicants were attempting to claim a composition comprising NAC and doxorubicin. However, applicants' claims are not composition claims.

The Examiner's inherency position would also be appropriate if applicants were claiming a process of administering a combination of doxorubicin and NAC to cancer patients in the later states of the disease, wherein metastasization has already taken place. However, this is not claimed.

Applicants have limited the claims to a narrow use not suggested by the prior art of record and not inherent in what was done in the Freeman and Doroshow references. Applicants claim administration of the combination to the narrow group of patients

who have primary tumors which are capable of metastasizing in the patient but have not yet metastasized.

The group of patients set forth in applicant's claims is a group of patients that would not normally be treated using doxorubicin. Hence, there would be no need to use N-acetyl-cysteine in combination with the not used doxorubicin and, accordingly, the claimed invention would not be anticipated by the prior art.

It is respectfully submitted that the rejection under 35 U.S.C. 102(b) is not tenable and should be withdrawn.

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Customer Number
22850

Norman F. Oblon
Attorney of Record
Registration No. 24,618

Tel: (703) 413-3000
Fax: (703) 413 -2220
(OSMMN 08/03)
NFO:JTG:aaf

John T. Goolkasian
Registration No. 26,142

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LEWIS LUNG CARCINOMA IN MICE AS AN EXPERIMENTAL THERAPY MODEL I. THE GROWTH KINETICS AND THE EFFECT OF TUMOR ON HOST*

by

WŁADYSŁAW BUDZYŃSKI

Department of Tumor Immunology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław

Some biologic characteristics of Lewis lung carcinoma (LL) which are relevant for the experimental protocols both for the single drug and combined treatment were examined. The studies included the influence of route and size of inoculum on the median survival time (MST) of tumor bearing mice, the growth kinetics of primary tumor and time of metastases appearance. In addition the effect of tumor growth on some host parameters as hematopoietic stem cells activity and on the spleen weights was tested.

Transplantable mouse tumors are nowadays the best but still not ideal screening system used both in evaluation of new anti-cancer drugs and in programming of different treatment strategies. Majority of anticancer drugs used presently in clinic were selected in mice bearing L1210 and P388 leukemias[1, 6]. These are exponentially, fast growing tumors with high proliferating fraction, which kill the host within few days[9, 14].

Drugs already selected by these models appeared to be effective in the treatment of patients mostly with leukemias and lymphomas. On the other hand, the solid tumors like e. g. lung cancer, gastrointestinal cancer are in majority of cases resistant to the treatment[2]. Thus, the great interest is focused on the slow growing solid mouse tumors as the screening models which could substitute the transplantable mouse leukemias in the selection of anticancer drugs active in human solid tumors[1, 6, 7, 8]. Such animals models include: Lewis lung carcinoma, B16 melanocarcinoma, MD-Madison lung carcinoma, 16/C mammary adenocarcinoma, C6-Colon 26 carcinoma, C8-Colon 38 carcinoma.

* This work was supported by National Cancer Program PR-6, Grant No. 11.
Abbreviations used: LL — Lewis lung carcinoma; MST — median survival time; CFU-s colony forming units.

The present study was undertaken with the aim to elaborate a new, more efficacious treatment method of LL to be introduced in the clinics. LL was discovered by Dr. Margaret Lewis in 1951[12]. Histologic examination revealed it is anaplastic epidermoid carcinoma[15] (Fig. 1).

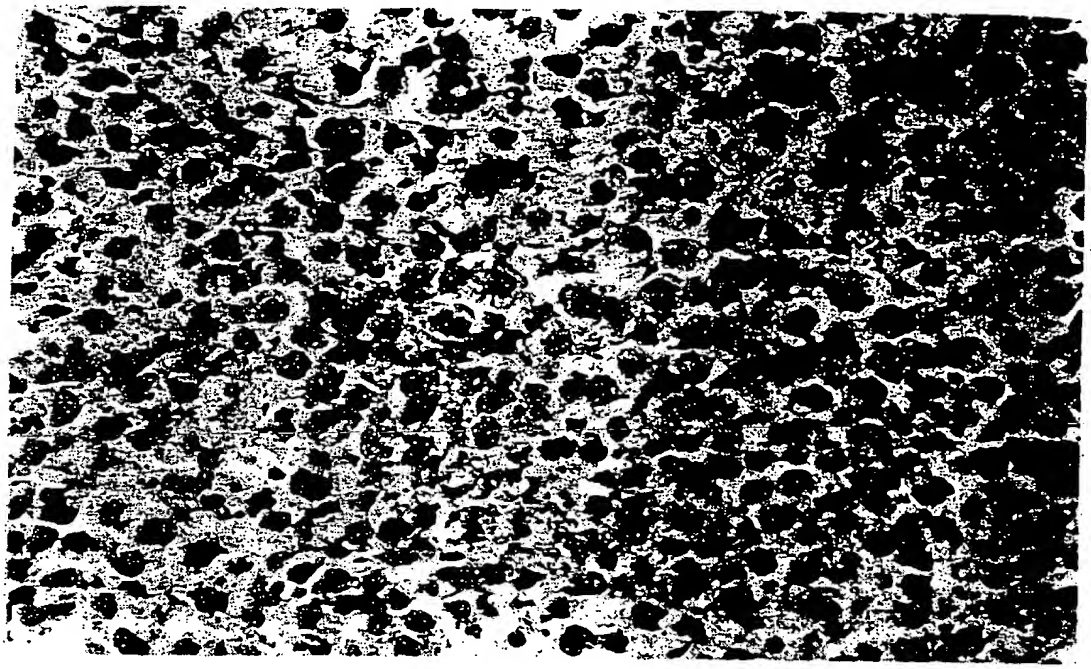

Fig. 1. LL cells taken from the primary tumor 8 days after s.c. transplantation. H. E. × 500.

MATERIALS AND METHODS

Mice. BDF_1 $(C57BL/6 \times DBA/2)F_1$ male mice, 2—3 months old were used in all experiments. The mice were obtained from Charles River Italia SpA.

Tumor. The tumor line used in the studies was obtained from Dr. I. Wodinsky (Arthur D. Little, Cambridge, Mass.). The tumor is maintained in our laboratory by serial s.c. transplantation in C57BL/6 mice.

The number of LL cells inoculation and the MST of mice. Asceptically removed tumors were homogenized and cell suspensions containing: 5×10^7/ml, 5×10^6/ml, 3.75×10^5/ml, $2{,}5 \times 10^5$/ml, 5×10^4/ml, 5×10^3/ml and 5×10^2/ml were made. The groups of BDF_1 mice were inoculated s. c. with 0.2 ml of these cell suspensions. The MST was evaluated.

Route of LL cells inoculation. Two milion of tumor cells which appeared to be the optimal size of inoculum was implanted s.c., i.m., and i.v. into BDF_1 mice. The MST for each group was determined.

Growth kinetics of LL and appearance of metastases. The primary tumors were excised and weighed every second day starting from day 2 to day 30. For examination of the micrometastases, the lungs were aseptically removed on the above mentioned days and inoculated s.c. with syringe into syngeneic recipients. At the same time the lungs from the paralleled group of mice were examined macroscopically and weighed.

Tumor development and bone marrow alterations and spleen weights

A. Evaluation of the bone marrow nucleated cells including colony forming units (CFU-s). Bone marrow suspension in Hank's medium was prepared by washing the marrow cells from the femur shafts of tumor bearing mice with a syringe and 20 gauge needle. The bone marrow nucleated cells were measured by counter (Coulter Electronics, Harpenden, Herts). The examinations were performed on days 1, 7, 14 and 21 after s.c. implantation of tumor cells. The number of colony forming units (CFU-s) was determined according to the spleen colony technique of Till and McCulloch[16]. The suspension of bone marrow nucleated cells was appropriately diluted in Hank's medium to obtain the final suspension 10^5 cells/ml. 0.5 ml of this suspension was injected into the tail vein of BDF_1 male mice which were exposed 24 hr earlier to 1000 R from Gammatron Co-60. Eight days later the recipients were killed and the spleens were fixed in Telleysniczky's fluid. The spleen colonies were counted (Fig. 2). The control mice were irradiated but not repopulated with bone marrow cells and the presence of the endogenous colonies was recorded.

B. Spleen weight. The BDF_1 mice were implanted s.c. with 2×10^6 tumor cells. The spleens from 4 animals were removed and weighed on days 0, 4, 8, 12, up to day 28.

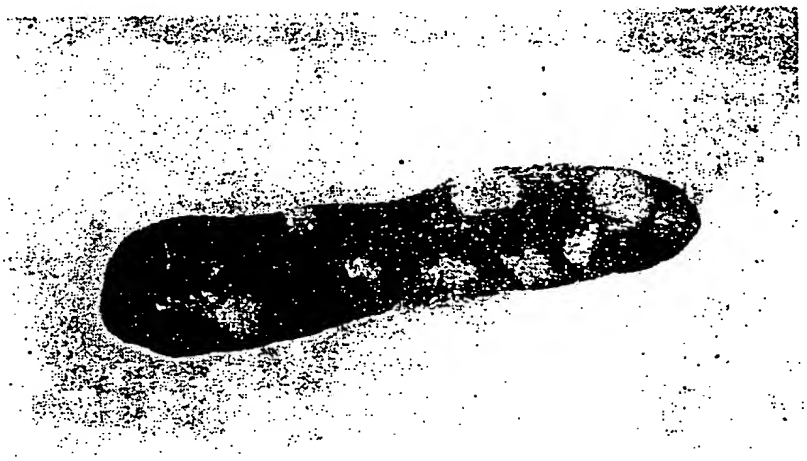

Fig. 2. Spleen colonies 8 days after 0.5×10^5 nucleated bone marrow cells i.v. injection.

RESULTS

The lower doses of inoculum (10^2, 10^3 and 10^4 tumor cells) appeared to be too low to induce the growth of tumor during 60 days of observation. After s. c. inoculation of 10^6 tumor cells the MST was 29 days while after inoculation of 10^7 cells per mouse, it appeared to be 19 days. Some "no takes" were observed in mice inoculated with 5×10^4, 7.5×10^4 and 10^5 cells. The results are summarized in Table 1. 2×10^6 tumor cells per mouse was accepted as an optimal size of inoculum and was applied in the experiments in which various routes of implantation were tested. After s.c. implantation of 2×10^6 tumor cells, 100% tumor "takes" was observed in 9 separate experiments. There were no spontaneous tumor regressions and no survivors. Median day of death was 28 with the range of medians from 22 to 35 days and a range of deaths in individual mice between 16—39 days. After i.m. implantation of 2×10^6 cells MST was day 22 and after i.v. inoculation — day 20. The

Table 1. MST of BDF_1 mice inoculated s.c. with various numbers of LL cells

Number of cells/inoculum	MST (days)	Tumor free survivors No/total
10^2	60	15/15
10^3	60	15/15
10^4	60	15/15
5×10^4	4 mice 60	4/10
7.5×10^4	2 mice 60	2/10
10^5	9 mice 60	9/30
10^6	29	0/15
10^7	19	0/15

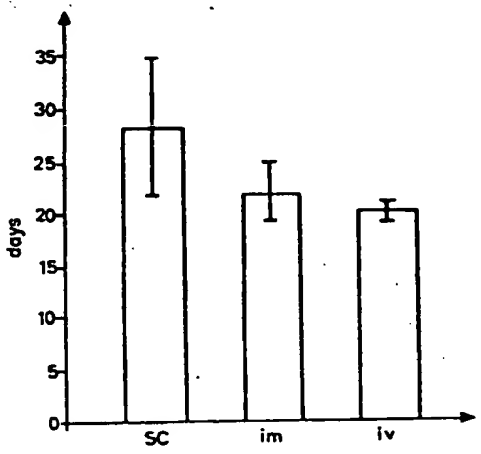

Fig. 3. MST of BDF_1 mice inoculated s.c., i.m., and i.v. with 2×10^6 LL cells.

data concerning MST depending of route of implantation are presented in Fig. 3. The growth of s.c. implanted LL cells is well described by a Gompertz function. The doubling time increases from 2 days for 500 mg to 3 days for a 1000 mg and 9.5 days when the tumor mass reaches 5000 mg (Fig. 4).

The increase of weight of lungs and spleens was observed in the course of tumor development. The lung weight increment was connected with the appearance of metastases. The micrometastases were detectable on day 8 when the average primary tumor weight was 548 mg and macroscopically visible appeared on day 16 after tumor cells implantation when the average primary tumor weight was 3308 mg (Table 2). On day 28 average weight of lungs was about 4 times higher than in control groups.

The size of mouse lungs 30 days after s.c. tumor cells inoculation is presented in Fig. 5A as compared to control (Fig. 5B).

As indicated in Fig. 6 the tumor development has a slight depressive effect on both the number of nucleated bone marrow cells and on hematopoietics stem cells (CFU-s). Twenty four hours after tumor cells implantation the slight decrease

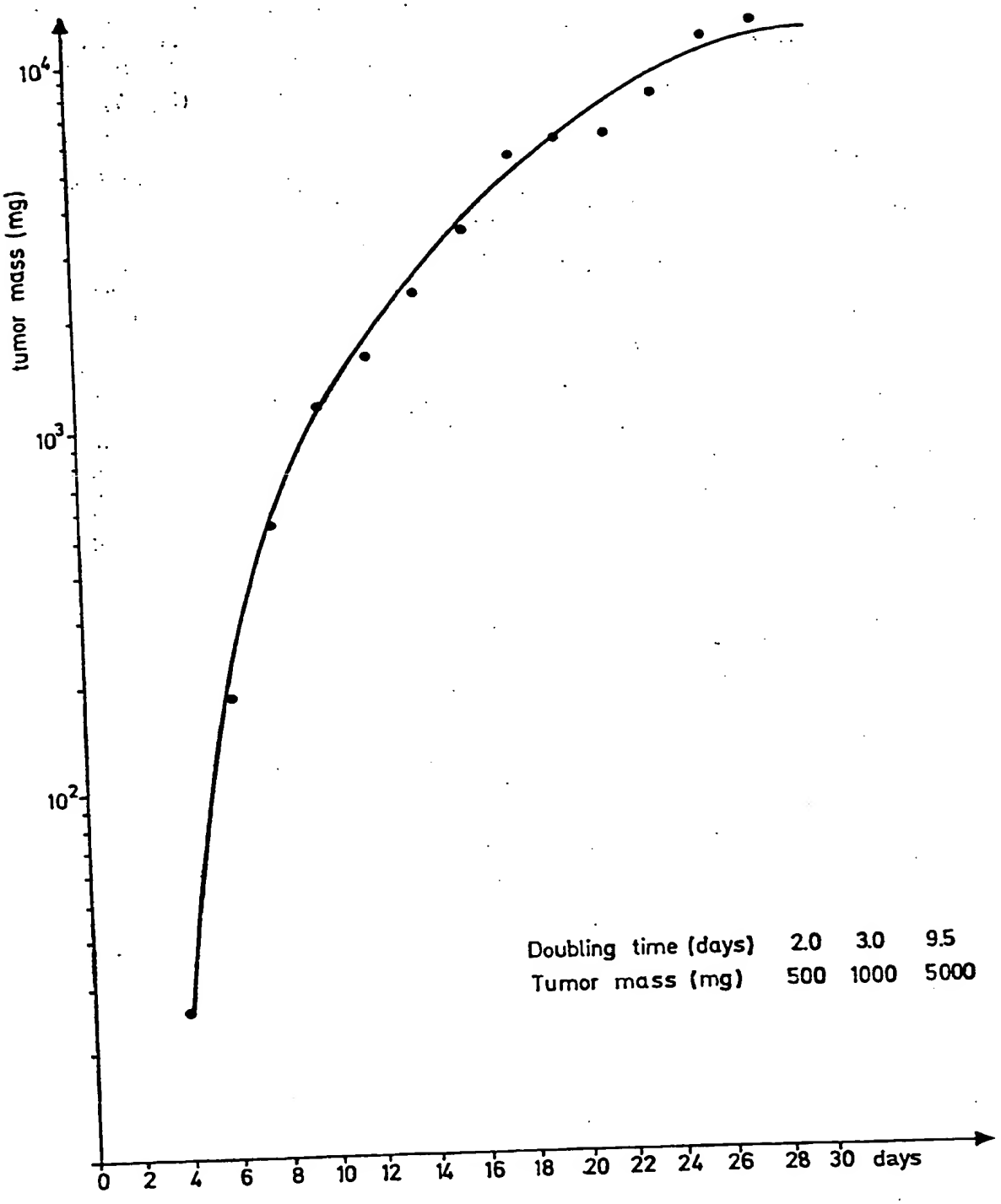

Fig. 4. Growth curve for LL implanted s.c.

(20%) in bone marrow cellularity was observed. Similar decrease (26%) in the number of CFU-s was observed 24 hours and 7 days after tumor cells implantation.

The increase in weight of the spleens was observed during the tumor development. On day 12 the median spleen weights were about 3 times higher than in control and these weights were maintained until the deaths of mice (Fig. 7).

Table 2. Microscopically and macroscopically detectable lung metastases of LL after s.c. inoculation of 2×10^6 tumor cells into BDF_1 mice

Days postimplant	Average primary tumor weight mg ± SD	Average lung weight mg ± SD	Microscopical metastases	Macroscopical metastases
0	0	130± 8	—	—
4	26±5	139±17	—	—
8	548±98	154±19	+	—
12	1533±369	176±46	+	—
16	3308±768	174±45	+	+
20	5877±572	170±13	+	+
24	7435±974	223±70	+	+
28	10838±862	588±77	+	+

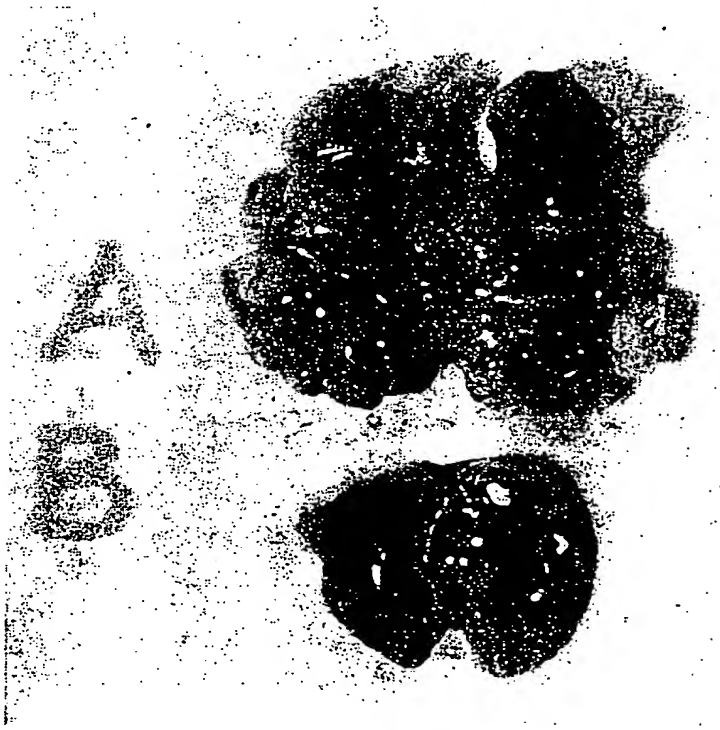

Fig. 5. Mouse lungs 30 days after 2×10^6 tumor cells s.c. implantation (A) as compared to control (B).

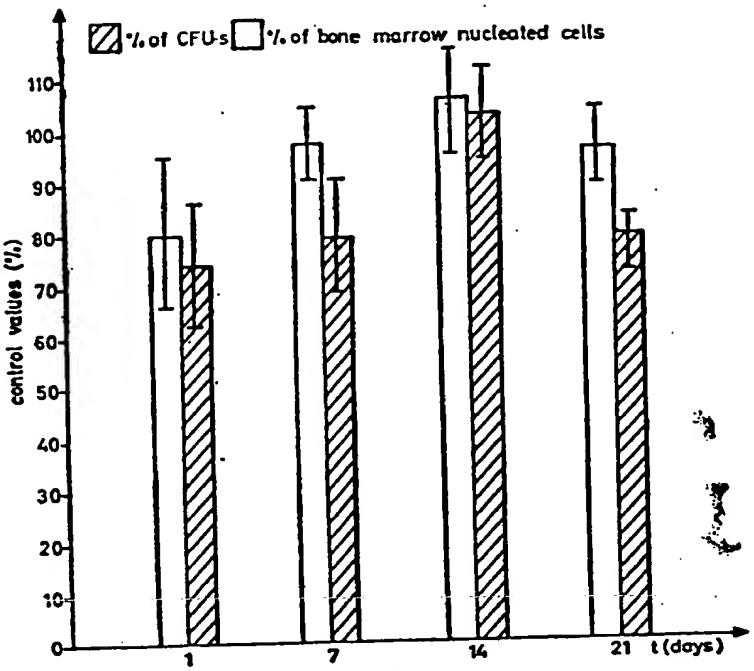

Fig. 6. Bone marrow nucleated cells and CFU content per femur at different days of LL development, expressed as a fraction of control.

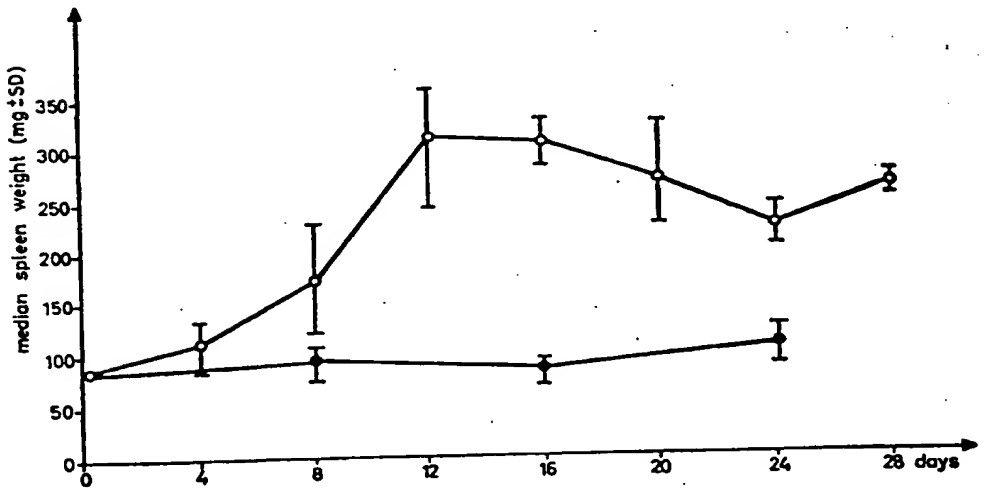

Fig. 7. Changes in splenic mass on different days of tumor development (o), as compared to control (•).

DISCUSSION

Despite of over 40 anticancer drugs available and used in the treatment of patients with various types of tumors, there is an urgent need for more effective drugs

to be applied in human cancer especially of lung, gastrointestinal tract and brain. This was the assumption to develop new animal tumor models which will select the drugs active for this group of patients. Ll seems to be a model which accomplished this purpose. It differs from mouse leukemia in slower growth, metastases and in sensitivity to majority of anticancer drugs available nowadays in clinic, selected in leukemia system. The knowledge of its biologic characteristics ought to help in manipulation and control over the growth of tumor cell population and in elaboration of a new treatment protocols. The performed studies indicate the correlation between the number of s.c. cell implantation and the MST. The smaller sizes of tumor cell inoculum (10^5) caused in some cases the rejection of tumors. The presence of the transplantation antigens in this tumor was demonstrated by FOGEL et al.[5], who also found the difference between the antigens on primary tumor cells and on the cells from lung metastases. Other observation suggesting the immunogenicity of this tumor is the enlargement of spleen weights in tumor-bearing mice. Maximal increase in the spleen weight on day 17 after 5×10^5 tumor cells implantation was described by TREVAS et al. and was interpreted as a result of host immunologic response to the tumor cells[17]. Similarly, the spleen enlargement was observed in mice implanted with other transplantable tumors as e.g. Sa 180, B16, mammary adenocarcinoma (unpublished data).

In the studies on growth kinetics, the MST after 2×10^6 tumor cells s.c. injection was found on day 28. Similar result was obtained by SIMPSON-HERREN et al.[14], a little shorter time (25 days) was observed by MAYO[12]. The growth of primary tumor is well described by Gompertz function which well characterizes the growth of majority of transplantable tumors[4]. It is possible to measure the doubling time from the obtained curve (Fig. 4). The doubling time increases from 2.0 days for the tumor of 500 mg to 9.5 days when the tumor mass reaches 5000 mg.

Thus, the higher tumor mass the longer doubling time. The growth of tumor is influenced by many factors. The studies on LL cells showed that when the mean tumor weight increases from 0.3 to 2.7 g, the growth fraction decreases from 35% to 18%, thus, the percent of cells in cycle affects the doubling time[12]. In some tumors the cell cycle can be prolonged during the tumor development[9]. The other factor is the death of some tumor cells because of anoxia, immunologic response of the host or chemotherapy[3]. Summing up, the tumor growth is a resultant of different factors which can influence its kinetics.

The tumor growth can be evaluated by the dynamics of metastases appearance. The appearance of macroscopically visible metastases on day 16 is consistent with the observation of other authors. The microscopic metastases appeared earlier. They were detected already on day 8. The studies of other authors showed the appearance of micrometastases on day 2 in single cases and in 100% cases on day 6[12]. The differences between the results can be explained by different test procedures used by this author.

The influence of tumor growth on hematopoietic stem cells was tested as a parameter of the tumor — host relationship. It is known that the administration of anticancer drugs to patients or experimental animals results in the elimination

of cells from hematopoietic system. The question is if the tumor itself reveals an influence on normal bone marrow cells. Twenty four hours after 2×10^6 tumor cell inoculation the slight decrease of nucleated bone marrow cell (about 20%) and CFU-s (about 26%) was observed. Similar decrement in the number of CFU-s was observed on day 7. In agreement with this observation are these of KHAITOV et al. who described the decrease in the number of CFU-s in mice bearing transplantable Ca755 or mammary adenocarcinoma[10]. Similar observation was reported by LALA who studied this phenomenon in mice implanted with Ehrlich carcinoma or Ta-3/St/ tumor[11]. The decrement of CFU-s in the bone marrow was caused by the migration of CFU-s from bone marrow to the spleen after tumor cells inoculation. These changes in the bone marrow cellularity appeared at the different time and were dependent on the type of tumor and strain of mice. Similar decrease in the number of CFU-s was observed after different antigen stimulation[13].

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